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The object of this Bulletin is to provide a guide to medical science and thought in Britain, and it consists mainly of summaries of a representative selection of British papers on subjects of medical interest. Any material appearing in the Bulletin may be published without fee, but acknowledgment of the source, by addition of the initial letters BMB followed by the serial numbers of the items selected, would be appreciated.

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SPECIAL CONTRIBUTION

RECENT ADVANCES IN BACTERIOLOGICAL METHODS

by J. C. CRUICKSHANK, M.B., CH.B., D.T.M., DIP. BACT.

Although there is a constant flow of contributions to our bacteriological knowledge, the most striking advances follow in the wake of some new development in technique. It is of interest, therefore, to consider some of the more important methods which have been described in recent years. An observer returning to the medical laboratory after an interval of a decade would note changes in two principal directions. He would be struck by the efficiency of the modern selective culture media for the isolation of bacteria. Further, he would find that the bacteriologist is no longer content to identify an organism as a particular bacterial species but, in dealing with many important groups, regards it as essential to carry the identification a stage further by determining the subgroup or type to which the strain belongs. These types, to which reference will be made later, are a stable character of the organisms, so that the typing methods make it possible to trace the migrations of a strain and frequently to detect the probable source of an infection or an epidemic.

Each of these lines of advance is well exemplified in the investigation of the intestinal pathogens. Many new culture media have been described for their isolation and, while there is little to choose between the best of these, it is clear that they are so vastly superior to media such as MacConkey or Endo agar that the latter may almost be regarded as obsolete for this purpose. In selectivity, satisfactory differentiation of colonies, and rapidity of growth, Leifson's medium, or some modification such as that of Hynes (1942), or S.S. (Salmonella-Shigella) agar, has proved outstanding for the isolation of organisms of the typhoid-paratyphoid, Salmonella, and dysentery groups. These media, which contain sodium desoxycholate, inhibit the growth of *Bact. coli* and Gram-positive bacteria. The bismuth-sulphite-iron medium of Wilson and Blair is still held in high regard for the isolation of organisms of the enteric group, especially *Bact. typhosum*. It is the routine in most laboratories to culture fæces and urine from suspected enteric cases directly on desoxycholate and bismuth-sulphite media, and also to sow a fairly heavy inoculum into a fluid enrichment medium such as tetrathionate broth, from which, after incubation, subculture is made, again on to one or both of the solid selective media. Extensive experience has shown that by this technique the pathogen will seldom escape detection in infective material.

With a series of fæcal specimens from dysentery cases inoculated in parallel on Hynes' medium and on MacConkey agar, twice the number of isolations of Flexner's and Sonne's bacilli were made on the former in acute cases and six times the number in convalescence. A tellurite-iron citrate-neutral red agar, with or without rosolic acid, has also proved valuable for the dysentery group (Wilson & Blair, 1941). The data obtained from such investigations have necessitated the revision of previous conceptions in respect to carrier rates, the duration of excretion of the organism in convalescence, and the control of chemotherapy.

In the Salmonella group the pioneer work of antigenic analysis by Kauffmann and Bruce-White has been followed up, and many new Salmonella types have been recognised. In this field, however, the most far-reaching discovery was the demonstration by Felix (1934) of the Vi or virulence antigen of the typhoid bacillus. This is a labile antigen, associated with full virulence of the organism, found in freshly isolated strains, but readily lost on subculture. It is reasonable to think that an effective antityphoid vaccine should be prepared from a typhoid strain possessing Vi antigen, and laboratory trials and, more recently, experiences in the field confirm that this is so. As heat and most of the antiseptics usually employed to preserve vaccines cause damage to the Vi antigen, Felix (1941) prepared a vaccine which was sterilised and preserved with alcohol. This vaccine has been shown to be safe and to produce a satisfactory response of Vi antibodies in inoculated subjects. Antityphoid serum for therapeutic use should also contain Vi antibodies. A further development of Felix's work was his discovery that the blood of typhoid carriers almost always contains Vi antibodies; the Vi agglutination test has thus become a valuable means of narrowing the field in the search for carriers, and the test is, moreover, independent of intermittency of excretion of typhoid bacilli (Felix, 1938).

In 1938 and subsequent years, Craigie & Yen (1938), who had been working with a bacteriophage which was specific in its lytic action on Vi strains of typhoid bacilli, reported that, by propagation of this phage on individual strains, they were able to produce Vi phages specific in their action on these particular strains. These specific phages could thus be used to identify the strains, and typing of typhoid bacilli became possible. Craigie described 18 types and subtypes to which a number of new ones have now been added. A fresh weapon was placed in the hands of the epidemiologist, and already there have come from Britain and America a number of fascinating accounts * of the way in which a strain of a certain phage-type responsible for an epidemic or a few scattered cases has been traced back to its ultimate source. The technique of phage-typing is a specialised one usually carried out in a central reference laboratory, but facilities for its performance should be available to all public health investigators.

In the bacteriological diagnosis of diphtheria it is now acknowledged that it is necessary to use a selective medium containing tellurite. The laboratory which relies, as in the past, solely on Löffler's serum is likely to miss some of the acute cases and to fail to detect the diphtheria bacillus in a large number of contacts, carriers and convalescents. The ideal tellurite medium would be easily prepared, would grow typical colonies of all diphtheria strains in 24 hours and would inhibit other organisms. Further, the microscopic morphology of the organisms would be readily recognisable. The perfect medium has not yet been devised, but of the various formulæ which approach it more or less closely, that of Hoyle (1941) is one of the most popular. This medium contains blood, lysed by saponin or other means, and is specially successful when prepared with a meat-extract base by a method which avoids excessive heating (Johnstone & Zinnemann, 1943). The good tellurite medium also permits easy identification, by differences in colony form, of the *gravis*, *intermedius* and *mitis* types of diphtheria bacilli, a differentiation confirmed by subculture into certain other media including starch, which is fermented only by the *gravis* type. These types, which all produce the same toxin, were first described by Anderson, Happold, McLeod & Thomson (1931), in Leeds, where the *gravis* type was always, and the *intermedius* type almost always, found to be virulent to guinea-pigs. These two types are associated as a rule in man with more severe infections than the *mitis* type, which may, however, also cause severe and even fatal infections. Typing of diphtheria bacilli has frequently assisted in the detection of carriers responsible for outbreaks and has already afforded valuable information concerning cross-infection in hospital wards. Typing data collected over long periods throughout the world promise ultimately to give a better understanding of the epidemiology of diphtheria and the long-term relations of the organism to the human herd.

Hæmolytic streptococcal infections in the past presented many problems to the investigator. Many of these are still unsolved, but the technical advances of the last 15 years (Ministry of Health, 1939) have made it possible to distinguish the findings which are significant. Streptococci isolated from swabs or infective material must first be shown to be true β-hæmolytic streptococci by the ability of a young fluid culture to lyse a suspension of horse erythrocytes. Organisms giving a positive soluble hæmolysin test may then be classified into one or other of the serological groups described by Lancefield. This test, performance of which presents no great difficulty, consists in extracting from the organisms by acid, or by Fuller's formamide method, a carbohydrate substance which gives a precipitation reaction in the presence of the appropriate group antiserum. Thirteen groups designated A to M are recognised and, while certain of the other groups are not without pathogenicity for man, the hæmolytic streptococci which are of major importance in human infections belong to group A. Group A organisms are, however, not infrequently found in the normal throat, and identification must be carried a step further. By means of agglutination tests with absorbed sera, technically rather more difficult than the grouping procedure, Griffith defined 23 types within

* [*see, for example, BMB* 70]

group A. New types are constantly being recognised, and certain types are found in frequent association with certain forms of infection, so that one may refer, for example, to a "common scarlet fever type."

Lancefield and Griffith may be said to have played the same role as an earlier bacteriologist of whom it was said that he shook the tree for his followers to pick up the apples. Their methods rapidly led to new discoveries. It was confirmed, as the clinician had already suspected, that the same hæmolytic streptococcus may give rise to various manifestations in a single community. The search for, and isolation of, carriers was put on a more rational basis. New information relating to cross-infections in surgical and fever wards became available. Nowhere was the value of the new techniques more strikingly shown than in the careful investigation into puerperal fever by the workers at *Queen Charlotte's Hospital* in London. Only by the typing method could it have been proved that endogenous infection, *i.e.* infection of the placental site by organisms already present in the genital passages at the commencement of labour, is almost negligible as a cause of puerperal fever, and that the important sources of infection are to be found in the attendants or other contacts, or more rarely in the upper respiratory passages of the patient herself.

It was natural to hope that some equally satisfactory classification might be possible in the staphylococcus group. *Staph. aureus* infections are important to the surgeon, and in skin conditions, especially in children, and it is being increasingly recognised that certain strains are responsible for a frequent form of bacterial food poisoning. In view of the ubiquity of staphylococci it is desirable that there should be some means of identifying pathogenic strains and, if possible, of typing them. The coagulase test (Medical Research Council, 1943), which depends on the ability of a strain to form a coagulum in human or rabbit plasma, is the most reliable of the suggested methods of identifying potentially pathogenic and toxigenic strains of *Staph. aureus*. Although typing of staphylococci has not so far given such spectacular results as that of streptococci, the method has proved of some value and will probably be developed. It was studied by Cowan (1939) who, by observing agglutination of suspensions by absorbed sera on a slide, was able to divide strains into three main types and a number of additional subtypes. In an investigation suggested by the isolation of staphylococci from the inside of surgeons' gloves after operations, he and his colleagues showed that carriage of the organism on the hands was related to chronic carriage of the same type in the nose. Attempts by Fisk (1942) to apply phage-susceptibility of staphylococci for purposes of identification are still in an experimental stage.

The present war, like the last one, has provided a stimulus to the study of the organisms of the gas-gangrene group. The best methods of using the existing techniques have been studied and defined (Medical Research Council, 1943). The examination of anærobic bacteria on a large scale has been hampered by the need of special apparatus, and it is of interest to note the development of methods of growing these organisms in the presence of air. This may be done by adding to fluid media, which must be heated immediately before use, 0·1 to 0·2% of agar and certain reducing substances such as ascorbic or thioglycollic acid. Vigorous growth of anærobes can also be obtained in ordinary broth or peptone water by the addition of a small strip of sheet-iron.* Characteristic changes produced in certain of the iron media provide additional means of bacterial identification.

The overcrowding which occurs in time of war also stimulates research on airborne infections, at any time a matter of high importance owing to the extreme difficulty of controlling such disease. The accurate sampling of bacteria in the air presents a considerable problem, since the older method of exposing open plates of culture media for a prolonged period resulted in the selective capture of the heavier dust particles. This has been overcome by the slit-sampler devised by Bourdillon, Lidwell & Thomas (1941). Air is drawn through a slit by means of a vacuum pump and made to impinge on a rotating plate of culture medium, if necessary a medium containing gentian violet which will permit the selective growth of streptococci indicative of respiratory pollution. The collection of data from samples thus obtained during short periods under different conditions has put these studies on a new footing and made possible the adequate control of experimental work on aerial disinfection by hypochlorites, hexyl-resorcinol, propylene-glycol and other agents.

The amazing discoveries in the sphere of chemotherapy have been a close concern of the bacteriologist, who has himself not been idle in the search for substances with bactericidal or bacteriostatic action. Penicillin, which may prove, when the problem of production in adequate quantity is solved, to be one of the most valuable of these agents, is the discovery of a bacteriologist, and other substances extracted from bacteria and fungi are constantly being investigated (Waksman, 1941).

The widespread use of the drugs of the sulphonamide group has caused the bacteriologist to modify certain of his methods. He now frequently receives blood or other material for culture containing a sufficiently high concentration of the drug to inhibit growth of the organisms *in vitro*. To counteract this, para-aminobenzoic acid is added to the culture medium; this substance, which is an essential growth factor for many organisms, is utilised by means of an enzyme, the action of which would otherwise be diverted by the chemically similar sulphonamide.

The methods noted above are some of those which are already yielding results of practical importance in the diagnosis and control of disease. The past few years have also seen the appearance of apparatus of great interest to workers in various branches of science which will undoubtedly further our theoretical knowledge of bacteriology and immunology. The electron microscope which extends resolving power to a present potential limit of about 50 Ångström units has already given new information concerning the morphology of bacteria and viruses, and the character and thickness of films of antibody on sensitised bacteria. It is probable also that the study of viruses, phages and antibody complexes will be greatly advanced by the use of the new high-speed centrifuges, the largest of which, the Svedberg centrifuge, runs at speeds up to 65,000 revolutions per minute.

* [*See also BMB* **147**]

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[2] [*See BMB* **76**]

Dr. J. C. Cruickshank was a member of the West African Medical Staff in Gambia for a number of years before becoming Lecturer in Bacteriology at the London School of Hygiene and Tropical Medicine. He is at present in charge of the Exeter laboratory of the Emergency Public Health Laboratory Service [*which is described in BMB* **64**].

At the School of Hygiene Dr. Cruickshank was one of a team engaged in the isolation of immunising fractions from bacteria by chemical means. Fractions effective in the prophylaxis of the infection in mice were obtained from the whooping-cough bacillus (with G. G. Freeman). His published work includes papers on the so-called Bacterium typhi flavum, on certain antigenic components of the Salmonellas, and the chemotherapy of experimental pertussis and anthrax infections.

BACTERIOLOGICAL METHODS AND THEIR APPLICATION

A SIMPLE "CHOCOLATE"-STARCH MEDIUM FOR THE RAPID TYPE DIFFERENTIATION OF *C. DIPHTHERIÆ*

by R. Hodgson, R. M. Heggie & P. L. Sutherland, *Journal of Pathology & Bacteriology*, **55**, 199–204, April 1943

The epidemiological value of differentiating the types of diphtheria bacilli is now widely recognised. A provisional opinion in respect to the type can usually be given from the colonial appearance on the primary culture-plate on which the swab was inoculated, and the type is finally established by subculture into other media, including starch and broth tubes. The present workers, from the Public Health Laboratory of Wakefield, England, have devised a method for the simpler and more rapid identification of diphtheria types by subculture from the primary plate to a single solid medium, the result being obtained from the appearances on this medium after 24 hours' incubation.

Three stock solutions are required: (*a*) 2 % starch solution, sterilised by autoclaving; (*b*) 1 % cystine solution, as used in Clauberg's medium, boiled immediately before use; (*c*) 2 % nutrient agar. To 100 ml. of melted agar, cooled to 60° C., add 10 ml. of horse blood. Mix and place in a water-bath at 75° C. for 10 minutes. Cool to 50° C. and add the following, mixing well after each addition: 1 ml. of cystine solution, 20 ml. of horse serum, 15 ml. of starch solution. Pour plates fairly thickly. It is advisable to store the blood and serum in the ice-chest for some days before use, to overcome the possibility that the diastase in these substances may cause hydrolysis of the starch. It is to be noted that the medium is not a selective one suitable for primary culture.

Colonies from the primary plate are spread over a square centimetre of the medium, as many as 12 cultures being seeded on to a single plate. After 24 hours' incubation, cultures of *intermedius* type are easily recognisable by the appearance described by the Leeds workers, Gordon & Higginbottom (1942). They present a fine growth with a tendency to form individual colonies, and produce a definite dull greenish discoloration in the medium. The plates are then flooded with Jensen's iodine (1 % iodine in 2 % potassium iodide), the excess being drained off after a few seconds. Two minutes later, identification of *gravis* and *mitis* growths can be made. The *gravis* cultures, which were creamy white, opaque and confluent, with a dryish appearance in reflected light, are now bright yellow and surrounded by a distinct halo, the medium immediately below and around the growth being transparent against the dark chocolate colour of the rest of the medium. These appearances were originally described by Anderson *et al.* of Leeds University (1931). *Gravis* cultures tend later to flake off from the medium. *Mitis* growths, which were also creamy white but less dense and more glistening than those of *gravis* type, become yellowish-green on treatment with the iodine, but have no halo. Subcultures may still be made after addition of the iodine.

With 120 strains examined by this method, and by the usual biochemical reactions, there was agreement as to type in 118, the remaining two strains, which gave a *mitis* type of growth, being non-starch-fermenting strains of atypical character.

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[1] [*see BMB* **32**]

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SLIDE CULTURE METHOD FOR TUBERCLE BACILLI

by K. S. Rosenberg, *Lancet*, **1**, 615–616, 15/5/43

Tubercle bacilli are unlikely to be found in direct films of tuberculous material unless it contains at least 10^5 organisms per cm^3. Culture on Loewenstein's medium can detect bacilli in material containing as few as 10 organisms per cm^3., but the result is probably not available for 3 weeks. With the method now described positive results could be obtained in a week with material containing 10^3–10^5 tubercle bacilli per cm^3.

The principle of the method, originally described by Pryce (1941) working with the *Emergency Medical Service* at an English sanatorium, consists in treating with acid a film of the material spread on a slide. Contaminating organisms having thus been destroyed, the film is washed, immersed in a suitable fluid culture medium, and incubated. The present worker, at another sanatorium in the same *Service*, uses as culture medium Kirchner's serum-salt mixture. This consists of equal parts of normal human serum and a synthetic medium: di-sodium orthophosphate 3 g.; mono-potassium orthophosphate 4 g.; magnesium sulphate 0·6 g.; asparagin 2·5 g.; glycerol 20 cm^3.; distilled water to 1 litre.

Further modifications of the method have been made, the author's technique being as follows. One-ounce [about 28 cm^3.] bottles with perforated screw-caps and rubber washers are filled with water and autoclaved. The suspected material is spread on microscopic slides which have been divided longitudinally. After drying in the incubator, the films are treated with 6 % H_2SO_4 for 45 minutes in a sterile staining rack with a glass lid. Two slides are then placed back to back in the bottles of water for 10 minutes. A needle attached to a water-pump is thrust through the cap, and the water is withdrawn and replaced by culture medium. The bottles are incubated for one week, then the slides are stained by Ziehl-Neelsen's method. Where tubercle bacilli have grown, the clumps are usually visible with the low power of the microscope.

Of a series of sputa positive by direct examination, 95 % showed a visible growth in 6 days. Of 105 sputa negative by direct examination, 11 showed good growth, 3 showed isolated bacilli, and 3 were contaminated. If non-purulent sputa, none of which were positive by any method, are disregarded, the positive findings by the slide-culture method in this series rise from 14 % to 24 %. The tubercle bacilli in this trial were all of human type.

There is thus evidence that this simple and inexpensive procedure is valuable for the detection of tubercle bacilli within a week in a considerable proportion of sputa negative on direct examination.

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147

IRON MEDIA FOR CULTIVATION OF ANÆROBIC BACTERIA IN AIR

by N. J. Hayward & A. A. Miles, *Lancet*, **1**, 645–646, 22/5/43

The adequate study of anærobic bacteria is often hampered by the scarcity of anærobic jars. Certain culture media of high reducing intensity, such as thioglycollate-agar-broth, permit the growth of these organisms under ærobic conditions, but there is need for a more stable and easily prepared agent. Spray (1936) suggested the use of a strip of iron and the present authors, working in the Department of Bacteriology of *University College* Medical School, London, where Miles is Professor of Bacteriology, have extended the method.

The fluid media, peptone water, "sugar" media or milk, preferably in narrow test-tubes, are heated in a boiling water-bath to drive off the air, and cooled quickly. Strips of sheet iron (no. 26 gauge, measuring 3 × 25 mm.) are dropped into each tube. The strips, which are sterilised by flaming immediately before use, or dry-sterilised and stored in screw-capped bottles, can be recovered and used repeatedly. They were found more effective than iron filings.

On incubation a precipitate appears on the iron and at the bottom of the tube. Certain anærobes produce blackening of the iron and the medium is darkened. If a fermentable sugar is present, the acid produced prevents this blackening. These appearances may give additional aid in the identification of bacterial species.

Peptone water containing the strip was converted into a medium which induced vigorous growth from small inocula of the most fastidious anærobes, including *Cl. tetani* and *Cl. œdematiens*. The species tested included numerous strains of 11 species of Clostridia, and strains of *Fusiformis*

and anærobic streptococci. All grew well and their fermentation reactions, tested in iron-peptone-water sugar media, were found correct.

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148

SLIDE-TEST FOR COAGULASE-POSITIVE STAPHYLOCOCCI

by B. Cadness-Graves, R. Williams, G. J. Harper & A. A. Miles, *Lancet*, **1**, 736–738, 12/6/43

The most reliable laboratory test for the identification of potentially pathogenic strains of *Staph. aureus* is the coagulase test. This test, which may take 24 hours, depends on the property of these strains to coagulate citrated plasma. It has been suggested that these coagulase-positive potentially pathogenic strains be called *Staph. pyogenes* (Cruickshank, 1937). It had previously been noted by Much (1908) and by Birch-Hirschfeld (1934) that coagulase-positive cocci were rapidly clumped when suspended in plasma. If this occurred with all coagulase-positive strains and did not occur with other cocci, much time would be saved, since the clumping test occupies only a few seconds. The present workers report the results of a study of the correlation between the coagulase and the clumping tests. Dr. Cadness-Graves is a pathologist to the *Emergency Medical Service*, Miles is Professor of Bacteriology at *University College* (London), and the other two workers belong to a *Medical Research Council* Unit at Birmingham Accident Hospital.

In the first series of tests, made by one practised observer on 442 strains of staphylococci from various sources, there was agreement between the tests with 440 strains, of which 280 were positive and 160 negative. The remaining two strains gave a feebly positive coagulase test and a negative clumping test. No clumping occurred with any of the other common bacteria tested. A solution of fibrinogen could be used in place of plasma for either test.

In a second series, observations were made by three workers on 860 strains, 558 of which gave a positive coagulase test. A record was made of the rapidity of clumping, from which it was clear that late or doubtful clumping appearing after 20 seconds was not a reliable indicator of *Staph. pyogenes*. Regarding these strains as negative, a positive clumping test was found to indicate 87 % of strains of *Staph. pyogenes*. Coagulase-negative strains do not give a positive clumping test. The authors suggest that, with increased experience, results as clear-cut as those in the first series would be obtained.

The recommended technique for the rapid test is as follows: A small drop of water is placed on each of two slides with a loop of 2 mm. diameter. The colony to be tested is rubbed carefully into one drop so as to make a homogeneous *dense* suspension. A small quantity is transferred to the drop on the other slide to make a film. A 1-mm. loop of fresh human plasma is mixed rapidly with the suspension on the first slide. If clumping occurs within 10 seconds, a presumptive diagnosis of *Staph. pyogenes* may be made, confirmed by the appearance of the stained smear. If no clumping occurs, and the stained smear shows gram-positive cocci, a tube of broth is inoculated for a coagulase test by one of the accepted techniques. Though late clumping is suggestive, it must not be used as a basis of identification.

It is estimated that the use of this rapid method will reduce the number of coagulase tests required by about 40 %.

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TYPING OF PARATYPHOID B BACILLI BY MEANS OF Vi BACTERIOPHAGE: A Report to the Medical Research Council

by A. Felix & B. R. Callow, *British Medical Journal*, **2**, 127–130, 31/7/43

The typing of typhoid bacilli by means of specific Vi bacteriophages has provided the epidemiologist with a valuable method of tracing the source of infections.[1] The authors, working with the *Emergency Public Health Laboratory Service*, now show in the present paper, which is a Report to the *Medical Research Council*, that the same technique may be applied to the study of paratyphoid B.

Felix had previously shown that *Bact. paratyphosum* B has a heat-labile Vi antigen similar in nature to that of the typhoid bacillus, and all of the many strains examined in the course of the work reported here possessed this antigen. The phages encountered in the early part of the investigation proved to be O-phages, active against the O or somatic antigen of the bacillus. These phages, which lysed all strains of *Bact. paratyphosum* B and also those Salmonellas possessing the same O antigen, could not be induced to become specific for any particular strain.

Ultimately Vi phages were obtained and it was found that paratyphoid B strains were either lysed by all of these phages or were resistant to all phages. No further progress was made until the occurrence of a small outbreak at Ipswich. It was found possible to adapt one of the original Vi phages which was then able to lyse the previously resistant Ipswich strains. The original Vi phage was known as type 1, the new one as type 2, and a later type, adapted in the same way, as type 3a. Type 2 and type 3a phages are able to lyse type 1 bacilli as well as their own specific type. Another subtype, 3b, was later discovered. Strains of paratyphoid B bacilli which cannot be typed with any of the four Vi-phages are said to belong to group Z. Only 7 % of 714 strains could not be typed. This is in contrast to the work on the typhoid bacillus, in which Felix used 22 Vi phages and found 15·9 % of strains untypable.

As with the typhoid bacillus, carriers repeatedly examined are always found to carry the same type of organism, and cases infected from a single source are found to be excreting the same type.

The remainder of the paper gives an outline of the types of *Bact. paratyphosum* B found in the outbreaks in Great Britain in recent years. Large epidemics occurred in 1941 and these were all due to type 1.

[1 see *B.M.B.* **64**, **69** & **70** for accounts of this method of typing typhoid bacilli.]

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A NOTE ON THE VALUE OF PHAGE TYPING IN THE INVESTIGATION OF AN OUTBREAK OF PARATYPHOID B FEVER

by J. R. Hutchinson, *British Medical Journal*, **2**, 130, 31/7/43

The author, who is an epidemiologist of the British Ministry of Health, gives the first account of the use in the field of the bacteriophage method of typing paratyphoid B bacilli. Twelve cases were reported over a short period in the town of Ipswich. Ten of these were excreting type 2 and the other two type 1 bacilli. All the persons infected with type 2 organisms obtained confectionery from one shop, in which the source of infection was found in the person of an unattended case. The two excreters of type 1 bacilli had no dealings with this shop. Other cases which had occurred in the rural districts outside Ipswich fitted into the same picture, all those infected with type 2 bacilli being customers of the shop in question, and the excreters of type 1 bacilli having no contact with this source.

The complete agreement between the bacteriological and epidemiological findings thus illustrates the way in which the method is likely to prove of great value to the investigator.

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TYPHUS RICKETTSIAL AGGLUTINATION TESTS IN THE MIDDLE EAST FORCES AND EGYPT

by C. E. van Rooyen & W. G. C. Bearcroft, *Edinburgh Medical Journal*, **50**, 257–272, May 1943

The Weil-Felix reaction has been of great practical value in the laboratory diagnosis of the typhus group of fevers; but, although the results of agglutination of different O strains of *B. proteus* by the sera of patients suffering from rickettsial infections indicates differences in the causal organisms, the diseases within the group cannot be clearly differentiated by the Weil-Felix reaction. Now that modern techniques enable relatively pure suspensions of rickettsiæ to be prepared in the laboratory, the use of such suspensions in serological tests has already extended our knowledge of the relationship of the various types of human rickettsial infections to each other.

In this paper, the authors review the recent literature on this subject and publish their own observations on the sera of typhus cases encountered in the Middle East. They used five antigens in each test; three of the antigens were the standard Proteus OX_{19}, OX_2 and OXK suspensions and the other two were pure suspensions of epidemic and murine strains of typhus rickettsiae which had been prepared by Dr. James Craigie of Toronto University. Sera were examined from 23 cases of typhus among civilians in Egypt (Cairo and Tanta), and from 50 cases among British and Allied troops in Palestine, Syria, Iraq and Egypt, while the sera of 100 healthy persons and of 74 cases of other infections were examined as controls.

The results are set out in tabular form. There was close correlation between the results of the Weil-Felix and rickettsial agglutination tests. None of the sera from normal individuals or from patients suffering from diseases other than typhus gave false positive results with the rickettsial suspensions. Although the rickettsial agglutination reactions were not so easy to read as those of the Weil-Felix tests they served to distinguish between louse-borne epidemic typhus and the flea-borne murine type of infection. With the sera from cases of the epidemic disease, cross-agglutination to low titre occurred with the suspensions of murine rickettsiae, the ratio of titres with the homologous to the heterologous rickettsiae being approximately 3 to 1; in the case of murine typhus the corresponding ratio was roughly 10 to 1.

The incidence of typhus during 1942 was not high in the Middle East but the epidemic form tended to occur in Cairo, in the winter months, among the civil population. The murine form was more frequently met with among the troops, and the cases in Syria, Palestine and the Suez Canal Zone tended to be of this type.

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CANCER AND RADIOTHERAPY

SELECTED PAPERS FROM THE *ROYAL CANCER HOSPITAL (FREE)* AND THE *CHESTER BEATTY RESEARCH INSTITUTE*, Volume I, 1935–1939, London:

I. The Problem of the Surgical Treatment of Cancer of the Rectum, by W. E. Miles (1939), *Amer. J. Surg.*, **46,** 26–39; II. The Pathology, Diagnosis, and Treatment of Hashimoto's Disease (Struma lymphomatosa), by C. A. Joll (1939), *Brit. J. Surg.*, **27,** 351–389; III. A Clinico-Pathological Classification of Cancer of the Breast, by R. W. Raven (1939), *Brit. med. J.*, **1,** 611–616; IV. Multiple Metastatic Tumours in the Brain Arising from Primary Bronchial Carcinoma, by W. E. C. Dickson & C. Worster-Drought (1936), *J. Neurol. Psychopath.*, **16,** 289–320; V. Multiple Meningeal and Perineural Tumours with Analogous Changes in the Glia and Ependyma (Neurofibroblastomatosis), by C. Worster-Drought, W. E. C. Dickson & W. H. McMenemey (1937), *Brain*, **60,** 85–117; VI. The Significance of the Röntgen, by W. V. Mayneord (1937), *Acta of the Internat. Union against Cancer*, **2,** 271–282; VII. The Use of X-Rays and Gamma Rays in Medicine, by W. V. Mayneord (1939), *Reports on Progress in Physics*, **5,** 284–301; VIII. The "Quality" of High Voltage Radiations—**1.**, by W. V. Mayneord & J. E. Roberts (1935), *Brit. J. Radiol.*, **8,** 341–364; IX. The "Quality" of High Voltage Radiations—**2.**, by J. R. Clarkson & W. V. Mayneord (1939), *Brit. J. Radiol.*, **12,** 168–180; X. Measurements of Low-Voltage X-Rays (Chaoul Technique), by W. V. Mayneord (1936), *Brit. J. Radiol.*, **9,** 215–237; XI. On Depth Doses from Teleradium Units, by W. V. Mayneord & J. Honeyburne (1938), *Brit. J. Radiol.*, **11,** 741–754; XII. A Preliminary Note on Low-Voltage X-Ray Therapy, by J. M. W. Morison, D. Hugo & W. V. Mayneord (1935), *Brit. med. J.*, **2,** 783–788; XIII. Short-Distance Low-Voltage X-Ray Therapy, by P. A. Flood & D. W. Smithers (1939), *Brit. J. Radiol.*, **12,** 462–485; XIV. The Rapid Production of Tumours by Two New Hydrocarbons, by W. E. Bachmann, E. L. Kennaway & N. M. Kennaway (1938), *Yale J. Biol. Med.*, **11,** 97–102; XV. Experiments on the Chemotherapy of Cancer: 2.—The Effect of Aldehydes and Glucosides, by E. Boyland & E. H. Mawson (1938), *Biochem. J.*, **32,** 1982–1987; XVI. Experiments on the Chemotherapy of Cancer: 3.—The Independence of Tissue Respiration and Glycolysis and the Growth Rate of Tumours, by E. Boyland & M. E. Boyland (1939), *Biochem. J.*, **33,** 618–621; XVII. Gall Stones and Cancer: A Problem of Aetiology with Special Reference to the Role of Irritation, by H. Burrows (1939), *Brit. J. Surg.*, **27,** 166–168; XVIII. Chemical Compounds as Carcinogenic Agents, by J. W. Cook, G. A. D. Haslewood, C. L. Hewett, I. Hieger, E. L. Kennaway & W. V. Mayneord (1937), *Amer. J. Cancer*, **29,** 219–259; XIX. Chemical Compounds as Carcinogenic Agents: First Supplementary Report:—Literature of 1937, by J. W. Cook & E. L. Kennaway (1938), *Amer. J. Cancer*, **33,** 50–97; XX. Photo-Oxides of Carcinogenic Hydrocarbons, by J. W. Cook, R. Martin & E. M. F. Roe (1939), *Nature*, **143,** 1020–1021; XXI. The Influence of Carcinogenic Compounds and Related Substances on the Rate of Growth of Spontaneous Tumours of the Mouse, by A. Haddow (1938), *J. Path. Bact.*, **47,** 567–579; XXII. The Influence of Carcinogenic Substances on Sarcomata Induced by the Same and Other Compounds, by A. Haddow (1938), *J. Path. Bact.*, **47,** 581–591; XXIII. The Biological Characters of Spontaneous Tumours of the Mouse, with Special Reference to Rate of Growth, by A. Haddow (1938), *J. Path. Bact.*, **47,** 553–565; XXIV. Cellular Inhibition and the Origin of Cancer, by A. Haddow (1938), *Acta of the Internat. Union against Cancer*, **3,** 342–353; XXV. The Association of Carcinogenicity and Growth-Inhibitory Power in the Polycyclic Hydrocarbons and other Substances, by A. Haddow & A. M. Robinson (1939), *Proc. roy. Soc. B.*, **127,** 277–287; XXVI. The Relative Potency of Carcinogenic Compounds, by J. Iball (1939), *Amer. J. Cancer*, **35,** 188–190; XXVII. Bile Production in Tumour-Bearing Mice and the Use of Bile in Filtration, by L. D. Parsons (1939), *Nature*, **144,** 75–76; XXVIII. Changes in the Lymph Glands of Tumour-Bearing Mice, by L. D. Parsons (1938), *J. Path. Bact.*, **47,** 501–523; XXIX. The Action of some *Endo*succinic Acids derived from Polycyclic Hydrocarbons on the Red Blood Corpuscles of the Mouse, by F. L. Warren (1939), *Biochem. J.*, **33,** 165–169; XXX. Estimations of Iron in the Lymph Glands of Mice during Treatment with a Carcinogenic Compound, by F. L. Warren (1939), *Biochem. J.*, **33,** 729–733.

[*These Papers, now collected together in one bound volume, have been selected for their outstanding interest from those published in various journals by members of the Staff of the Royal Cancer Hospital (Free) during the years* 1935 *to* 1939.

They are concerned partly with clinical aspects, partly with physical problems of determining the dose of radiation needed for the successful treatment of malignant disease, and partly with work on experimental carcinogenesis.

The following is a composite review by three different writers. It has been divided into Clinical, Physical and Experimental Sections.]

A. *CLINICAL*

I. This paper opens with a discussion of the method of spread of cancer of the rectum. There are three distinct paths:

(i) by direct extension through continuity of tissue,
(ii) through the venous system,
(iii) through the lymphatic system.

Spread by the lymphatic system is much the most important, and to deal with this satisfactorily an abdomino-perineal operation is needed.

The one-stage abdomino-perineal operation practised by the author is described in detail with great clarity. The preparation of the patient receives attention and the importance of following exactly the instructions given is stressed. The technique of the operation is given under three headings: the abdominal, pelvic, and perineal portions. The position of the incision and the exposure are mentioned and then the actual operative procedure is divided into seven stages, with each stage so described that they can be followed exactly, from the first examination of the pelvic colon to the

completion of the operation by the removal of the isolated bowel and the opening of the proximal end of the pelvic colon. In conclusion, the author describes the methods of dressing and after-treatment.

II. This condition, about which there is considerable confusion, is clearly distinguished from other types of thyroid disease or neoplasia and it is pointed out that Hashimoto's name " Struma lymphomatosa " is undoubtedly correct. The condition with which it is most often confused, Riedel's Disease, is also described and numerous coloured illustrations bring out the difference between the two.

While it is true that the pathogenesis and ætiology are obscure, the clinical findings, together with the histological appearances, are sufficiently characteristic for diagnosis of the condition. The differential diagnosis by clinical signs and symptoms may be difficult. Length of history and size and type of goitre are important, and pressure effects and the presence or absence of myxœdema or hyperthyroidism must be looked for.

Treatment may be surgical for the relief of pressure symptoms, but probably radio-therapy will do all that is necessary. The prognosis in treated cases is good, but the possibility that myxœdema will supervene must be kept in mind.

III. The author proposes a new classification of carcinoma of the breast, founded on both clinical and pathological findings.

IV. The difficulty of distinguishing between metastases from bronchiogenic carcinoma and primary brain tumours remains very great, but increasing knowledge of the behaviour of carcinoma of the lung shows that this syndrome is not uncommon. Three types of cases have produced it:

(i) sclerosing bronchial carcinoma,
(ii) small growths in relation to the secondary bronchus,
(iii) massive primary in the periphery of the lung.

Such cases are illustrated by numerous photographs and photomicrographs. Metastases are found most often in the temporo-sphenoidal and frontal lobes, in the cerebellum, and also in the choroid plexuses, where their appearance may lead to an erroneous diagnosis of malignant papilloma of the choroid.

V. Two cases of this rare condition are fully described. Both ended fatally and, at *post mortem*, multiple tumours were found in the central nervous system and some on the peripheral nerves. Histologically, the tumours varied from meningioma to neurofibroma. The condition is described as neurofibroblastomatosis.

It is believed that this, like other multiple tumour syndromes, is due to a disturbance in the action of the evocators which stimulate and regulate the growth of tissues and organs. The authors advance certain theories as to the possible means by which perversion of the evocation fields at an early stage in embryonic life may result in multiple neoplasia.

B. *PHYSICAL*

VI. Calculations of the effective Atomic Numbers and X-ray absorption coefficients of a large number of biologically important substances, including carbohydrates and proteins, show that they will differ from air in their X-ray energy absorption. On the whole it may be said that the energy absorbed by soft tissues will be within 10 % or 15 % of that absorbed by air for wavelengths greater than 0·2Å: for shorter rays the agreement will be much closer. In the case of certain substances such as thyroxine, the absorption coefficient is high, but as these substances are present only in small amounts, the total effect is not more than a 7 % increase in energy absorption. Thus it is shown that the *roentgen* (for which a revised definition is proposed) is a very suitable unit for the measurement of short wave electromagnetic radiations, but warning is given that it would be unwise to deduce from the dose in *roentgens* the energy absorption in special regions.

The variation of radiation " quality " within a mass of tissues is described.

VII. Possible methods of dosage measurement are reviewed and the measurement, in terms of the most suitable unit (namely the *roentgen*) for X and gamma rays, by means of the parallel-plate chamber and of small enclosed chambers, described and discussed. The relationship between *roentgens* and the energy absorbed in human tissue is surveyed as in the preceding paper.

The production and uses of Isodose Curves are described and the reasons for the present trend towards higher voltage X-rays are discussed.

A survey of the methods of using radium therapeutically is made, with special reference to the many mathematical studies of source distribution. The radium " bomb " is described and a typical dose distribution is shown.

VIII. Of the three methods of " quality " specification, viz., the Equivalent Constant Potential, the Equivalent Wavelength, and the Half-Value Layer, the last is shown to be the most suitable for heterogeneous beams. The measurement of the absorption coefficients of certain light elements is suggested as a possible alternative.

The suitability of different metals as filter materials is investigated by spectrographic and ionisation measurements, and tin is shown to be superior to lead or copper for the 200–400 kv. range. Of various combinations of metals tried only a tin-tantallum filter showed any improvement over tin, whilst barium-copper has possibilities.

Tin is also considered to be the best material for Half-Value Layer measurements in the 200–400 kv. range.

IX. Two methods, one depending on the differential increase in photo-electric emission from the walls of metal and carbon ionisation chambers with increasing wavelength, and the other on the variation, with wavelength, of absorption in copper, are used to explore changes in " effective wavelength " of X-radiation within a scattering medium. Reasons for the final preference for the former method are given.

The effective wavelength increases with the field size and depth. At a depth, the value from 350 kv. primary radiation may be less than that for primary 250 kv. radiation.

A reduction in wavelength of the primary radiation alone is found with depth. The amounts of primary and secondary radiation at different depths are measured: the maximum amount of scattered radiation is reached at between 2 and 5 cm. deep; scatter may contribute up to 80 % of the dose at about 15 cm. deep.

The bearing of these results on the question of the relative biological effect of different X-ray wavelengths is emphasised.

X. Measurements of X-rays from the Siemens 60 kv. set, by means of a special dosage-rate meter employing a Lindemann electrometer and a very small chamber, are described. At 5 cm. focal skin distance the tube output is about 25 *r* per minute per milliampere, and the radiation has an average wavelength of about 0·32Å.

Percentage backscatter on the surface, percentage depth dose values and complete radiation distributions are given for a variety of fields. The small amount of radiation outside the geometric confines of the beam, and the considerable variation between centre and edge doses, are noted. Measurements of stray radiation around the tube are quoted.

XI. Using an experimental teleradium unit, the effects of variation of focal skin distance, filter thickness, and area of source, on dosage, have been investigated. The amount of soft radiation at the aperture increases with focal skin distance as does the percentage depth dose. A filter of 0·5 mm. lead plus 0·6 mm. brass gives the maximum depth dose improvement. For short (2–3 cm.) focal skin distance, increase in source area improves the percentage depth dose by some 20 %. No such change occurs for long (10 cm.) focal skin distance.

The depth dose cannot be accurately calculated by inverse square law alone. The effect of an apparent absorption coefficient of 0·025 $cm.^{-1}$ must be included for heavily filtered sources.

XII. An account is given of the first use of short distance low-voltage X-ray therapy in Britain, with a description of the methods used by Chaoul, the originator of this particular type of plant and the pioneer of this method of treatment.

The first 40 cases treated at the *Royal Cancer Hospital* are discussed.

XIII. The apparatus developed by Chaoul and Adam has been used in the treatment of 310 malignant and 190 non-malignant cases. Treatment by this method is now usually described as Contact Therapy.

For treatment to be successful, the lesion must be accessible and of reasonable depth. The tumour dose to be delivered in the time planned must be predetermined, and the volume treated must more than include the clinical limits of the tumour. Treatment may be by single or multiple fields and various arrangements are shown with photographs of

the tumours treated. The distribution of dosage is illustrated with various ingenious contrivances used in treatment. Tables show a high percentage of good results.

The Philips " Metalix " short distance low-voltage X-ray therapy apparatus is also described and a diagram is given.

C. *EXPERIMENTAL*

[Experimental Carcinogenesis, an outcome of the study of occupational cancer in man, became established as an essential weapon in the study of the cancer problem when, in 1915, Yamagiwa and Itchikawa succeeded in inducing tumours of the skin in rabbits by repeated applications of coal-tar. The full utilisation of this new technique depended, however, on the isolation and identification of the constituents of tar responsible for this action. The way in which this was achieved, by a fruitful collaboration between physicists, chemists and pathologists at the Research Institute of the *Royal Cancer Hospital*, under the leadership of Prof. E. L. Kennaway, represents one of the most fascinating chapters in modern cancer research. The adaptation, by Mayneord and Hieger, of fluorescence spectrography as a means of recognising carcinogenic fractions in tar, led to the isolation, from some 2 tons of pitch, of a few grammes of a highly carcinogenic crystalline product, which was subsequently identified by Cook and Hewett as the polycyclic hydrocarbon, 3 : 4-benzpyrene. The synthesis of several hundred related compounds, of widely varying carcinogenic potency, followed in due course, partly as an attempt to correlate chemical structure and carcinogenicity, and partly to investigate any possible relationship between these compounds and the sterols. In this development of the subject, the Research Institute of the *Royal Cancer Hospital* (subsequently constituted as the *Chester Beatty Research Institute*) continued to play a leading part.]

There are 17 papers on subjects connected with experimental cancer research. These include 2 reviews of the literature on chemical compounds as carcinogenic agents (XVIII, XIX), 5 publications on the inhibitory action of carcinogens on tumour growth (XXI–XXV), 4 on carcinogenesis by hydrocarbons and other agents (XIV, XVII, XX, XXVI), and 6 on various other aspects of the cancer problem (XV, XVI, XXVII–XXX).

In XVIII and XIX considerable space is devoted to the relationship between the chemical structure of polycyclic hydrocarbons and their carcinogenic potencies, while due attention is also given to such biological considerations as the mechanism of carcinogenesis ; local, systemic, and genetic factors influencing carcinogenesis ; and the systemic effects of carcinogens on normal and neoplastic tissues. Brief sections on histology and cytology, tissue culture, and viruses, in relation to carcinogenesis, complete the reviews. The scope of these reviews is wide, the bibliography (which includes 374 references) is accurate and comprehensive, and the presentation of the material is lucid ; criticism is sound though sparing, and speculation is moderate and cautious. These qualities, and the fact that the literature on the subject is being brought up to date by the authors in Supplementary Reports, render the reviews standard sources of reference for all scientific workers in cancer research.

An interesting development of the work on pure carcinogenic hydrocarbons was the observation by Haddow, in 1935, that, in addition to their carcinogenic action at the site of injection, these hydrocarbons had a profound inhibitory effect on the growth of tumours in other parts of the body. The importance of this discovery, both from the theoretical point of view, in regard to the mechanism of carcinogenesis, and from the practical angle, with a view to possible therapeutic application, prompted an extension of these investigations to many other hydrocarbons and their derivatives. The subjects of the five communications (XXI–XXV) on this aspect of the problem in the " Selected Papers " are indicated by the titles given at the head of this review. Paper XXV contains an account of the results of 348 experiments in which 96 different compounds were tested for their action on the growth both of transplanted tumours in mice and rats and of spontaneous tumours in mice. Paper XXIII is manifestly designed as a basis for the accurate assessment of the results of Haddow's inhibition experiments, while XXIV is a review of a rather speculative character, in which the results of tumour inhibition are considered in relation to the general nature of carcinogenesis. Impairment, rather than stimulation, of growth, is stressed as the primary influence of carcinogenic action, while the irreversibility of the neoplastic state is considered as characteristic of the established tumour cell. These factors are discussed in relation to the " Somatic Mutation Theory " of cancer.

The four papers on carcinogenesis comprise an account of the effects of 9 : 10-dimethyl- and 5 : 9 : 10-trimethyl-1 : 2-benzanthracene on the mouse's skin (XIV) ; a comparison of the potencies of 24 compounds (XXVI) ; a note on the question as to whether conversion of carcinogens into photo-oxides plays a preliminary part in their biological action (XX) ; and a description of unsuccessful attempts to induce tumours of the gall bladder in guinea-pigs (XVII).

Paper XV describes an extension of the work of Strong on the inhibitory effect of heptaldehyde on tumour growth. Of the aldehydes, ketones, and glucosides tested by these workers, citral and heptaldehyde were found to inhibit the growth of spontaneous tumours in mice. In Paper XVI, the interesting observation is made that there appears to be no correlation between the rate of growth of a tumour (whether growing normally or artificially inhibited) and its respiration and glycolysis values measured *in vitro*.

The subjects of the remaining papers (XXVII–XXX) are indicated by their titles. The " Selected Papers " form a valuable addition to the literature on experimental cancer, and the promise of a second volume, mentioned in the Foreword, is to be welcomed.

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EPITHELIAL TUMOURS OF THE BLADDER IN DOGS INDUCED BY PURE BETA-NAPHTHYLAMINE

by G. M. Bonser, *Journal of Pathology and Bacteriology*, **55**, 1–6, January, 1943

Since Rehn (1895) first demonstrated a causal relation between occupation in the synthetic dye industry and the development of tumours of the bladder, many hundreds of such cases have been reported in the literature. Knowledge of the incidence of this occupational disease, its geographical distribution, pathology, symptomatology and treatment, is adequately summarised in recent reviews on the subject (International Labour Office, 1921 ; Berenblum, 1932 ; Ferguson *et al.*, 1934 ; Hueper, 1938). The ætiology has for long been controversial. The finished dyes were clearly not responsible for the tumour formation in the bladder, and of the intermediate products used in the manufacture of the dyes, the four compounds most widely used in the industry—aniline, benzidine and alpha- and beta-naphthylamine—were considered to be the most likely causes of the disease. Final proof depended, however, on experimental evidence in animals.

The earlier experiments proved unsuccessful. Subsequently, both Schär (1930) and Perlmann & Staehler (1932) claimed to have induced tumours in rabbits with naphthylamines. The accompanying photomicrographs were, however, most unconvincing, and in a more extensive series of experiments by Berenblum & Bonser (1937), these results on rabbits could not be confirmed. The position was finally clarified by Hueper and his collaborators. They found that whereas the rabbit failed to develop tumours of the bladder following prolonged administration of beta-naphthylamine (Hueper, Briggs & Wolfe, 1938) several such tumours developed in dogs (Hueper, Wiley & Wolfe, 1938).

One problem remained undecided, however. These authors used a commercial preparation of beta-naphthylamine, and it was not possible to say whether the tumours were due to the action on the body of the naphthylamine itself or of some impurities present. This has now been established by the author of the present paper, who is Research Fellow at the Department of Experimental Pathology and Cancer Research at the University of Leeds. Using pure beta-naphthylamine, she succeeded in producing both benign and malignant tumours of the epithelial lining of the urinary bladder in 3 out of 4 dogs which were fed daily with 100–150 mg. of this substance, later increased to 700 mg. daily, for periods varying from 3 years and 8 months to 5 years. Malignancy was judged by invasion of the sub-epithelial tissues, including the musculature. Metastases did not occur. Other parts of the urinary tract (kidney, renal pelvis, ureters or urethra) were free from tumours.

This work finally establishes the fact that beta-naphthylamine produces tumours of the epithelium of the urinary

bladder, apart from any impurities which may be present in the product used in the synthetic dye industry.

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DISCUSSION ON THE TECHNIQUE OF RADIOTHERAPY

by B. W. Windeyer, C. Wood & R. Paterson, *Proceedings of the Royal Society of Medicine*, **36**, 261–270, March 1943

Radiotherapy is playing a part of increasing importance in the treatment of cancer of the larynx and pharynx, and three papers read at a discussion on the technique of radiotherapy held by the Section of Laryngology of the *Royal Society of Medicine* are here reported.

B. W. Windeyer, Professor of Radiotherapy at the *Middlesex Hospital*, London, gave an account of the fundamental biological and general technical principles underlying the treatment of malignant neoplasms by X-rays and gamma rays. Biologically both X and gamma radiations exercise a destructive effect on tissues; but a selective damaging effect also exists whereby some malignant tissues are destroyed by a smaller dose of radiation than that required to destroy surrounding normal tissue. This special vulnerability of tissues to radiation forms the basis of radiosensitivity. Some neoplasms such as lymphosarcoma, lymphoepithelioma and basal-cell carcinoma are naturally radiosensitive; but the majority of neoplasms are not particularly so, and special variations in technique are necessary for treatment to be successful.

In applying radiotherapy to the treatment of disease three main techniques have been employed:

(i) Multiple small dose technique. This is used for inflammatory conditions but has no place in the treatment of malignant disease

(ii) Single massive dose technique. This is of value in the treatment of small superficial malignancies of the skin; it is followed by severe reactions

(iii) Fractionated technique. This is the most useful X-ray technique for the treatment of malignant disease. The time factor is an important consideration, as the treatment must be designed and given as a single course made up of fractional doses given on successive days. The total treatment time should not exceed 30–35 days, otherwise the malignant cells may become immune to the effects of the radiation.

The value of protraction of the individual treatment by the use of a low dosage rate is not yet established. Meanwhile the use of a high dosage rate (50 *r*/min.) rather than a low one (5 *r*/min.) has obvious economical advantages.

The quality of X-ray beam used in the treatment of individual lesions requires variation according to the position of the tumour in the tissues. For superficial neoplasms low voltage X-rays (60–100 kv.) are very useful. Most of the tumours of interest to the laryngologist are situated at a limited depth below the skin surface and X-rays of high voltage (180–250 kv.) are adequate. Supervoltage X-rays (250 kv. or more) are of value in the treatment of more deeply seated tumours such as carcinoma of the thoracic œsophagus.

In planning treatment for the individual case of cancer attention to detail and accuracy are important. The number, size, position and direction of the fields will depend on the situation, extent and character of the lesion and the presence or absence of metastases. For highly radiosensitive tumours with widespread metastases a few large fields may be used. Squamous cell carcinoma, however, requires multiple small field methods with accurate beam direction. The dose required for each of the various types of tumour is not yet known. Success does not depend on the actual dose delivered to the neoplasm, but the reactions of the tissues to the dose given are also of the greatest importance. Radiotherapy for malignant disease of the mouth and throat causes severe local reactions and depresses the general condition of the patient. It should not be regarded as an easy alternative to surgery.

Dr. Constance Wood, Director of the Radiotherapeutic Research Unit at the *Hammersmith Hospital*, London, dealt with the technique of teleradium treatment of cancer of the head and neck. At this centre a 10 g. radium beam unit is used, and with the aid of a special directional caliper, accurate treatment can be given through small fields. Before undertaking teleradium treatment to the head and neck regions, all septic teeth should be removed and the gums should be allowed to heal. In some cases of cancer of the larynx and pharynx a preliminary tracheotomy may be necessary, although in certain cases radiation treatment may prevent the need for this operation. Treatment should be so planned that the primary disease and its probable lines of spread should be adequately and homogeneously irradiated. As the quantity of radiation tolerated by a patient is limited, it is an advantage when treating cancer of the oral cavity or pharynx to irradiate the primary growth through its areas of lymph drainage in the neck. During treatment the general condition of the patient requires careful attention. The diet is important in these cases, since the radiation reaction of the mucous membranes of the mouth and pharynx interferes with proper feeding. The calory intake should be controlled and the diet should be high in vitamin content. From graphical records of the daily progress of dosage and the general and local responses of the patient to treatment the following observations were made:

(i) The best results were observed when the dose was approximately 6–7,000 *r* given in 42 days

(ii) Chief among the general constitutional effects were loss of weight and diminution of the total leucocytes, mainly lymphocytes, of the blood count

(iii) Locally, membranous reaction is noted at the primary site at the end of a week, reaching its maximum in the fourth week, and then subsiding. The skin reaction occurs later than the mucous membrane reaction, often not until the third or fourth week.

Dr. Ralston Paterson, Director of the *Holt Radium Institute*, Manchester, discussed the radiotherapeutic treatment of cancer of the pharynx and larynx. The two main types of malignant neoplasm affecting the larynx and pharynx are squamous cell cancer and reticulo-endothelial growths. The distinction between these two types is important as the principles of treatment differ in each case.

(1) Squamous cell cancer. This is the commonest type and where cure is intended the fundamental need of treatment is to irradiate the smallest volume of tissue which will adequately include the whole tumour, to the highest dose which will be safely tolerated. The chief methods of treatment are external radiation by X-rays or teleradium, but the following radium procedures are of value:

(*a*) Finzi-Harmer fenestration implant for growths of the middle third of the vocal cord

(*b*) Sponge rubber applicators for superficial growths of the post-nasal space

(*c*) Radon seed implantation of the oropharynx

(*d*) Combined radium collar and radium bougie for limited growths of the hypopharynx.

Radiation is of value in the palliative treatment of squamous cell cancer because it can relieve symptoms and prolong life. Such treatment should not be radical; the dosage given should be below maximum and the treatment time should be short.

(2) Reticulo-endothelial growths. This term includes such varieties as lymphosarcoma, reticulum cell sarcoma, lympho-epithelioma, endothelioma and round cell sarcoma. These tumours have two common characteristics: they are radiosensitive and radioresoluble. By radiosensitive is meant that they disappear quickly under radiation, and by

radioresoluble that there is no local recurrence in the area treated. The advantages of radioresolubility are minimised because these tumours disseminate early and widely. In contrast to squamous cancer, large volumes of tissue require radiation even when the disease appears limited. Success depends on early treatment before general dissemination has occurred, and a radical dosage whereby the whole of a large volume of tissue is treated to the limits of tolerance. Wide field X-ray therapy provides the best method of treatment for the reticulo-endothelial growths; radium is not a satisfactory alternative.

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BEAM DIRECTION IN RADIOTHERAPY: *Symposium* by F. Ellis, C. W. Wilson, J. L. Dobbie, L. G. Grimmett & T. A. Green, *British Journal of Radiology*, **16**, 31–43, February 1943

This symposium contains papers read at a recent meeting of the Radiotherapy Section of the *Faculty of Radiologists*. The expression Beam Direction is used to describe all methods which make more exact the treatment of malignant tumours by means of converging beams of X and γ rays. The problem has two aspects: first, the exact localisation of the tumour, and, secondly, the correct application of the therapeutic radiation beams under fixed geometrical conditions. The methods which have been developed may deal with the second question only, or may deal with both in combination. Although, in general, deep-seated tumours are considered, others, such as breast tumours, may also be suitable for the application of similar ideas.

F. Ellis of the *Sheffield Radium Centre* discusses the requirements of tumour localisation, and classifies methods of treatment more fully described in subsequent contributions. Methods of localisation usually depend on rendering the tumour radiographically visible. Implanted radon seeds, soft-tissue shadows, barium swallowed or injected through a catheter, and tomographs, are used in appropriate sites. Even so, the total size of the tumour is a matter calling for judicious estimation.

Beam Direction may depend on the judgment of the therapist, who makes the necessary adjustments by eye on each occasion. Alternatively, mechanical means, sometimes depending on skin marks or fluoroscopy, may be used. The author refers to an instrument which he has planned but not yet executed, and which, he hopes, will make possible treatment in any plane. This is a device for directing a beam of X-rays by means of an arc attached to the patient. The author also refers to the possibilities of rotational therapy.

C. W. Wilson of the Physics Department of *Westminster Hospital*, London, points out that in order to produce a desirable distribution of radiation in an isolated deep volume of tissues, it may be necessary to direct the several beams employed not at the centre of such a volume but at some other (usually deeper) point. Such a case is illustrated in the treatment of carcinoma of the larynx by means of 3 teleradium beams whose central axes intersect at a point 2 cm. behind the larynx, thus superimposing the area of high dosage on the area occupied by the larynx itself. To ensure the correct application of the radium bomb in these predetermined positions, a bridge-like device rising from a base-board is placed over the neck, and carries wooden discs on which the radium bomb is located.

As an example of similar principles applied to X-ray therapy, the author considers a tumour of the lung and shows that, by careful consideration of the dose-contours, two fields may be used in place of three, with improvement of depth-dose.

J. L. Dobbie of the *Holt Radium Institute*, Manchester, describes and illustrates two different systems of X-ray Beam Direction. The first method involves the construction for each patient of a rigid envelope in the form of a cap, collar, or jacket, to fit the part containing the tumour. These envelopes may be made of plaster bandage or sheet plastic material. The tumour is localised within the envelope and a suitable arrangement of treating beams is decided. The points of entry of their central axes are marked by wax seatings to locate the X-ray tube applicator, and the points of emergence are marked by small holes. During treatment a device is fixed to the X-ray tube which carries a pointer lying in the central axis of the beam. The patient wears the envelope, the applicator is located on the wax seating, and the pointer must engage in the appropriate hole on the far side of the envelope.

The second method is preferred where compression is important. The apparatus consists either of a bridge-like protractor placed across the patient, and centred on the previously determined position of the tumour, or of a portion of such a protractor attached to the X-ray tube in such a way that the applicator can be advanced towards the centre of the protractor, which is kept fixed in the predetermined position of the tumour. In both cases the direction of the beam and the tumour depth are read directly on appropriate scales.

T. A. Green of the Radiotherapy Department, *St. Thomas's Hospital*, London, had previously (1937) described a "caliper" for use with a teleradium unit, and he now describes a similar design suitable for attachment to an X-ray tube. This device is used with skin marks showing points of entrance and emergence of a line through the tumour. During treatment the applicator is applied at one skin mark while a pointer carried in the central axis on a movable arm is made to coincide with the other skin mark.

When compression is to be applied an arrangement as determined by the caliper is first made. A fixed pointer is then brought up to a scale on the applicator. As the tube is lowered to effect compression, adjustment of the couch or tube is made to keep the stationary pointer on the scale, which then shows the amount of movement in the direction of the central axis of the applicator.

In addition to movement in the central axis, the pointer of the caliper is capable of regulated movement for a considerable distance round it, so that by applying the pointer to any part of the patient's surface, the relation of that part to the dose contours of the beam can be determined. This modification finds special application in breast treatments.

When the vertical depth of a tumour below a skin mark is known, the direction and length of any path from it to points on the patient's skin can be reconstructed in space by a device which erects on any selected point a vertical line equal to the known vertical depth below the skin mark, and thus allows a parallelogram to be completed by a line equal in length and direction to the line joining the selected skin point and the tumour. The author describes a useful instrument for this purpose, which is fully illustrated in the original paper.

A second method of deriving the same information for the same purpose is provided by an arc which can be held suspended over the patient with a vertical radius centred on the tumour at known depths. Other radii, which may be provided by a ray of light from a lamp sliding round the arc, indicate skin points at known directions and distances from the tumour.

In the "predetermined shape method" the tissues to be treated, say, breast or neck, are enclosed in a close-fitting open box suitably shaped, and having two, three, or more sides. The unoccupied space within the box is filled with packing, and treatment is applied through each side in turn. In this way, fixed treating conditions are ensured, and exact dosage can be worked out.

L. G. Grimmett describes a development of the teleradium caliper designed by Green. This consists of an electrical system incorporated in the emergent pointer which rings a bell if the patient moves, beyond certain small limits, from his correct position during treatment.

The original papers of this symposium are accompanied by drawings and photographs which contribute greatly to their interest.

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THE TELERADIUM TREATMENT OF INTRINSIC CANCER OF THE LARYNX

by M. Lederman & W. A. Mill, *Journal of Laryngology and Otology*, **57**, 471–488, November 1942

There are three main radiotherapeutic methods available for treating intrinsic cancer of the larynx. These are:

(1) High voltage X-ray therapy.
(2) Fenestration of the larynx and radium implantation (Finzi-Harmer operation).
(3) Teleradium therapy (Radium "bomb" or "beam" therapy).

Of these methods, the last is probably the least commonly practised. The authors who are, respectively, Radium

Therapist and Laryngologist to the *Royal Cancer Hospital*, London, report a series of 35 cases treated at this centre by a 5 gram and 1 gram teleradium unit.

Under the term intrinsic cancer of the larynx they include neoplasms arising on the true cords, false cords, and ventricle, and the anterior and posterior commissures. Most of the cases treated showed advanced disease, only four cases being suitable for laryngo-fissure or fenestration. Ten were suitable for laryngectomy; nine, while technically operable, were unsuitable for laryngectomy because of the general condition of the patient; three cases were unsuitable for any form of surgical treatment. The authors advise biopsy in all cases with two possible contra-indications: (1) a patient with respiratory embarrassment and œdema can often be saved from a tracheotomy by correct radiotherapeutic treatment, but this may be prevented by an ill-advised biopsy, and (2) it is sometimes technically difficult to perform direct laryngoscopy on patients with short necks and good teeth. The authors do not insist upon biopsy in such cases if the clinical diagnosis seems certain.

Teleradium treatment is planned according to the general condition of the patient and the extent of the local disease. Treatment is restricted to the larynx, and the cervical lymph node areas are treated only if metastases are suspected on clinical grounds. A small field, 3·5 to 5 cm. in diameter, is placed over each thyroid ala, and these are sometimes supplemented by a third anterior field placed over the *pomum adami*. Two fields are treated each day for 6 days per week, each field receiving 300–350 *r* per treatment. The dose given depends on the response seen in each individual case; generally an attempt is made to deliver a tumour dose of 6-7,000 *r* in 4–6 weeks.

The chief complications of radiotherapeutic treatment are:

(1) Œdema, which may be seen during or after treatment. Its significance depends on the time relationship. When observed before treatment is begun, it may be due to sepsis. It is always an important sign and has a marked influence on the course of the treatment.

(2) Necrosis of the larynx is now much less common than formerly, and can be classified as follows:

(*a*) Immediate necrosis, which shows itself soon after the completion of treatment, without an intervening stage of healing.
(*b*) Early necrosis, which occurs within six to twelve months after apparent healing.
(*c*) Late necrosis, which occurs after healing which has lasted for a year or more.

Although the time periods are arbitrary the descriptive terms are of significance. Immediate and early necroses are usually due to faulty technique or over-dosage and are avoidable. Late necrosis is due to post-radiation *endarteritis obliterans* for which there is no known means of direct prevention. There were three cases of laryngeal necrosis among the thirty-five treated in the present series. All were probably due to a fault in technique which has now been corrected, and for the last four years no case of laryngeal necrosis has been seen.

(3) The authors strongly deprecate the performance of a tracheotomy either before or during treatment, as such cases never seem to do well. This operation was found necessary after treatment in four cases. In two cases a tracheotomy had to be performed as a palliative measure because the disease was not eradicated, in the remaining two cases because of necrosis or œdema.

The following plan is recommended for the treatment of recurrences:

A. For post-operative local recurrence teleradium is indicated as a curative or palliative method, while for recurrences after radiotherapeutic treatment excisional surgery is the best method, if possible. A second radiotherapeutic treatment is dangerous and rarely successful.

B. Cervical lymph node metastases present problems in treatment which have not yet been satisfactorily solved. Where no previous treatment has been given, teleradium is advised for both operable and inoperable lymph node metastases. If metastases occur in a region previously treated by radiotherapy surgical excision is indicated, or if this is not possible implantation of radium needles or radon seeds may be of palliative value. Lymph node recurrences after surgical removal of cervical lymph node areas can occasionally be successfully treated by teleradium. Implantation of radium or radon is often the quickest and simplest way of dealing with these cases.

There is a marked scarcity of published results of radiotherapeutic treatment of intrinsic cancer of the larynx. This can be remedied only if more cases are sent to the radiotherapist. Statistically significant results cannot be deduced from the present small series of cases, but as an index of the value of teleradium treatment the authors point out that, of 15 previously untreated cases observed for five years after treatment, 7 are known to be alive and well, one who is now untraceable was known to have had two years' freedom from symptoms, and one further patient died of intercurrent disease, *post mortem* examination of the larynx showing no evidence of cancer.

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THE VALUE OF POST-OPERATIVE RADIOTHERAPY IN CARCINOMA OF THE BREAST

by R. McWhirter, *Edinburgh Medical Journal*, **50**, 193–207, April 1943

The results obtained by radical mastectomy for early breast cancer probably represent the peak of surgical achievement in the treatment of cancer. Yet mammary cancer is the cause of approximately 20 % of cancer deaths in women; and moreover this percentage is remorselessly increasing.

The author, who is Radiologist to the *Royal Infirmary*, Edinburgh, attempts to prove that post-operative radiotherapy is of significant value in the treatment of breast cancer. It is notoriously difficult to determine and compare accurately the results obtained in the treatment of cancer of the breast by different methods. A uniform system of classification of cases according to the stage of advancement of the disease is essential for such a comparison to be satisfactory. The author uses the following scheme:

Stage I. Growth confined to the breast. Involvement of an area of skin directly over the tumour does not affect the staging. Paget's disease of the nipple is regarded as belonging to Stage I in the absence of palpable lymph nodes.

Stage II. As Stage I, but with palpable mobile lymph nodes in the axilla.

Stage III. The growth extends beyond the *corpus mammæ*, either deeply to the underlying muscle or superficially to the skin, which is invaded over a large area in relation to the size of the breast. Axillary lymph nodes if palpable must be mobile.

Stage IV. The growth has extended outside the breast area, *i.e.* fixed axillary lymph nodes, complete fixation of tumour to chest wall, supraclavicular or cutaneous metastases, or metastases in opposite breast or distant organs.

Records of 1879 cases of cancer of the breast seen between 1930–1942 were available. Of these cases 30 % were Stage I, 17 % Stage II, 20 % Stage III, and 21 % Stage IV. The remaining 12 % were not staged.

The methods of treatment selected for comparison were (*a*) radical surgery alone, and (*b*) surgery (either local or radical) associated with a full course of post-operative X-ray therapy. The radiotherapeutic treatment was considered adequate with doses of 3,500–4,500 *r* given in 3–4 weeks. The area treated comprised the chest wall and axillary and supraclavicular lymph nodes on the affected side. Only the cases forming Stages I–III were used for investigation since it was found that survival for 5 years in the Stage IV cases was negligible, no matter what the method of treatment employed.

The three-year symptom-free rate was chosen as the basis for comparison, as it was found statistically that this and the five-year survival-rate were almost equal (38 % and 37 % respectively for 566 cases in Stages I, II, and III).

The effect of post-operative radiotherapy (i) on the local recurrence rate in the operative field, and (ii) when the axillary lymph nodes are diseased were analysed in detail.

(i) It was found that post-operative radiotherapy was effective in destroying cells left behind in the "treatable area," *i.e.* the chest wall, axilla and supraclavicular regions. The recurrence rate in the treatable area was reduced by 18–20 % in all Stages when post-operative radiotherapy was employed, and the three-year symptom-free rate was increased by 22 %, 32 % and 23 % in Stages I, II and III respectively by the same means.

(ii) In order to assess the effect of post-operative radiotherapy on the axillary lymph nodes, the cases were arranged into two groups, one without histological evidence of

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axillary lymph node invasion and the other where such invasion was present. The three-year symptom-free rate for radical surgery alone was 57 % when the lymph nodes were histologically negative and 24 % when the nodes were involved. The comparable figures where post-operative radiotherapy was employed were 91 % and 50 %. It would therefore seem that post-operative radiotherapy can be relied upon to destroy malignant cells left behind in the axilla, and as the complete removal of axillary lymph nodes by surgery is never an easy matter the author has considered the possibilities of treating cases by local removal of the breast, leaving the axilla untouched, coupled with a full course of post-operative radiotherapy. Even should this method prove no more effective thán the radical operation plus radiotherapy it is still preferable, as the operative mortality is reduced and there is less subsequent disability. From observations extending over a year the results of simple mastectomy and radiotherapy appear to be as good as those following the radical operation and radiotherapy. A note of warning, however, is necessary as this method is still on trial and should not be adopted in centres where effective post-operative radiotherapy is not available.

The paper concludes with observations on the need for the early diagnosis and treatment of breast cancer. The average delay occurring before treatment is instituted is 9 months, and is largely due to the present outlook and organisation of medical practice. Were this delay eliminated, 50 % of cases of breast cancer could be cured by modern methods of treatment.

BIOLOGICAL EFFECTS OF X-RADIATION

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EFFECT OF X-RAYS ON AQUEOUS SOLUTIONS OF BIOLOGICALLY ACTIVE COMPOUNDS

by W. M. Dale, *British Journal of Radiology*, **16,** 171–172, June 1943

This paper outlines a series of investigations (Dale, 1940; 1942; 1943; Dale, Meredith & Tweedie, 1943) from the *Christie Hospital and Holt Radium Institute*, Manchester, on the effect of X-rays on aqueous solutions of biologically active compounds.

On exposing a wide range of concentrations of solutions of the crystalline enzyme carboxypeptidase to X-rays it was found that, for instance, a dose of 50 *r* inactivated about 30 % of a dilute solution, whereas a dose of 100,000 *r* was required to produce the same percentage effect on a solution 340 times as concentrated. A dilute solution, therefore, can be almost completely destroyed by a small dose, when a concentrated solution exposed to the same dose would appear unaffected (radioresistant). This may be briefly called the " dilution effect."

The quantitative test showed that for a given percentage destruction and a given dose of X-rays the absolute amount of the enzyme destroyed is constant whatever the initial concentration. This result can only be understood by assuming an *indirect* action of the radiation causing the formation of an intermediate product (Fricke, 1934; Hopwood, 1940; Luria & Exner, 1941; Risse, 1930; Gray & Read, 1942) from the solvent (water) which then acts on the enzyme. A direct action (" hit " theory) would require that the amount of enzyme inactivated increases with increasing concentration (the number of effective hits being proportional to the concentration).

When carboxypeptidase is irradiated in the presence of its substrate no inactivation takes place. This " protection effect " was analysed in the case of the enzyme d-amino-acid oxidase, which can be split into the prosthetic group flavine-adenine-dinucleotide and its specific protein, both of which together are necessary for enzymatic activity. Each one of these components is radiosensitive, though not equally, and if mixed, after being irradiated separately, they yield the greatest inactivation, whereas irradiation after mixing shows the " protection effect."

On adding a great variety of other substances (amino acids, sugars, nucleic acids, etc.), one at a time, to either of the two enzyme components protection again took place, proving that no specific compound of changed radiosensitivity was the cause of this protection. Sodium chloride did not protect at all. When the concentration of the substance added was progressively decreased, a corresponding decrease of the protection occurred. It was thus possible to determine for a given dose of X-rays the concentration of any of the substances added which was needed to protect to 50 %.

The indirect action concept simultaneously explains the protection phenomenon. If the intermediate product encounters two or more different solutes, and if these solutes are capable of reacting with it, each one will share in the reaction according to its concentration and affinity, leaving a larger or smaller share for its partner.

The indirect mode of action of X-rays and its consequences —the dilution and protection phenomena—can be shown to hold also for acetylcholine as a representative of an organic, non-protein, biologically active substance entirely unrelated to enzymes.

According to these investigations radiosensitivity will depend:

(*a*) on the specific affinity between the substance in question and the intermediate product formed from water during irradiation
(*b*) on the affinity of other substances present at the same time (protection effect)
(*c*) on the relative concentrations (dilution effect)
(*d*) on the particular physiological action of these substances.

With regard to the biological effects of X-rays (Evans, Slaughter, Little & Faila, 1942) on the more complex living matter, the following points may be mentioned as being in agreement with the results of these *in vitro* experiments:

(i) The unspecific though graded response of living matter to irradiation finds its counterpart in the susceptibility of so great a variety of substances responding to radiation
(ii) The great radiosensitivity of " specific " proteins which, unsurpassed by any other substance in these investigations, is in marked contrast to the gross changes of viscosity, precipitation, etc., of unspecific proteins seen after very intensive irradiation
(iii) The fact that younger cells, and especially embryonic cells, are more radiosensitive than older ones (greater general water content), and also that dry seeds are more radioresistant than seeds soaked with water
(iv) The increase of radiosensitivity of certain phases of a cell in a state of evolution in contrast to that of fully-developed long-lived cells. Here the unequal distribution of solutes enhanced by processes of mitosis and division offers the chance for the dilution and protection phenomena to come into play.

The investigations stress the importance of the indirect action theory of biological effects of radiation, in particular the radiosensitivity of enzymes if examined in suitable conditions. Earlier investigators, unaware of the protection and dilution phenomena, have used too impure and too concentrated solutions and have therefore been led to the erroneous belief that enzymes are too radioresistant to account for biological radiation effects.

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